Shape matters: relevance of carapace for brachyuran crab invaders

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Acknowledgements

Authors want to acknowledge the staff of the Biological Reference Collections (CBR) of the Institut de Ciències del Mar (ICM-CSIC) of Barcelona (Francisco J. Olivas and Ricardo Santos) for their help in the identification, separation and transferring of the samples used in the present study.

Abstract

The increasing trend of biological invasions is one of the most concerning threats for ecosystems functioning. The identification of optimal characters determining the invasive potential of non-native species has always been a challenge in conservation studies. Morphological features can be used as a good proxy to address the invasive success in fish species, assuming that anatomical differences in comparison to native species can provide to newcomers ecological opportunities increasing their probability to become successful. Considering this, the present study constitutes the first applicative attempt of a model based on geometric morphometrics to demonstrate the importance of the carapace shape in the invasive ability in marine decapod crustaceans. The study was performed on the native brachyuran community of Alfacs Bay, in the Ebro Delta, the largest estuarine zone along the north-western Mediterranean, in which two recently established non-native crab species coexist: *Dyspanopeus sayi* and *Callinectes sapidus*. Results suggested that invaders with extreme carapace traits located peripherally in the community morphospace, such as *C. sapidus*, usually possess ecological advantages contributing to understand their success. Conversely, intermediate morphologies within the morphospace, such as *D. sayi*, imply ecological overlapping with native species reducing their community relevance. Besides, we also assessed the effect on the community morphospace of a hypothetical future invasive event from another crab species (*Portunus segnis*) with high probabilities to also colonize the community. Our outcomes confirm that the morphometric approach could be an alternative tool for assessing the potential ability of invasive crab species. However, further studies at different spatial and temporal scales, including additional traits and quantitative data from invasions, would be necessary to confirm the efficacy and usefulness of the methodology.

Keywords

Invasive crabs · Carapace shape · Geometric morphometric analysis · Morphospace · Ecological competition · Estuarine community

Introduction

Within the current broadly accepted context of global change, multiple human-associated phenomena altering marine ecosystems at different levels of organization have already been identified (Harley et al. 2006; Halpern et al. 2008; Hoegh-Guldberg and Bruno 2010). Direct (e.g., fishing overexploitation, pollution or habitat loss) and indirect (e.g., global warming, ocean acidification or transport of ballast water) anthropogenic activities are intensively affecting the dynamics of biological communities, causing shifts in geographical distribution range and abundance of species (Parmesan and Yohe 2003; Perry et al. 2005; González et al. 2017) and changes in population dynamics of species affecting their recruitment, dispersion and performance at different life stages (Cury et al. 2008; Pörtner and Farrell 2008; Hidalgo et al. 2011) or in the biodiversity and structure of communities (Hughes et al. 2003; Philippart et al. 2011; Wernberg et al. 2013). However, one of the most concerning events, both for the scientific community and society, is the occurrence and establishment of alien invasive species in native communities, due to its impact on the ecosystem functioning and by their potential economic and ecological costs on ecosystem services (Harris and Tyrrell 2001; Stachowicz et al. 2002; Karatayev et al. 2009; Katsanevakis et al. 2014). Most studies related with the effects of biological invasions have been focused on the assessment of their ecological consequences on native communities (Grosholz 2002; Simberloff et al. 2013) or in the understanding of causes, vectors, pathways and mechanisms of invasive processes (including introduction, colonization, establishment and spread, see Walther et al. 2009) in order to, at larger extent, identify the optimal and common traits of successful invaders and attempt to predict their invasive potential (Kolar and Lodge 2001; Ficetola et al. 2007).

In the Mediterranean Sea, there are up to 45 different known species of allocthonous brachyuran crabs (Galil et al. 2002; Galil 2011), most of them inhabiting the benthic or demersal domain from sublittoral, littoral and estuarine zones, areas especially susceptible to biological invasions due to their high productivity and strong physicochemical fluctuations (Grosholz 2002; Lejeusne et al. 2014). Among them, some of the most common and better established in the western Mediterranean are native from western Atlantic waters, as the case of the American blue crab *Callinectes sapidus* (Rathbun, 1896) (with first reports detected in 2012 in the Ebro Delta, Castejón and Guerao 2013), and the Say mud crab *Dyspanopeus sayi* (Smith, 1869) (first record in 2005 in the Ebro Delta, Schubart et al. 2012). Both are euryhaline and eurythermal species native from the western Atlantic Ocean inhabiting shallow estuaries and coastal lagoons (Williams 1984; Nizinski 2003; Nehring 2011). They expanded eastwards probably through human-induced actions and they are currently widely expanded along the eastern Atlantic and most of the Mediterranean Sea (Froglia and Speranza 1993; Micu et al. 2010; Nehring 2011). Especially worrisome is the case of *C. sapidus*, since its wide environmental tolerance and adaptation, aggressive competition for resources, large size, high fecundity rates and large dispersal capacity (Hill et al. 1989; Nehring 2011; Mancinelli et al. 2013) turn him a successful invader representing a serious threat for native ecosystems. In fact, presently it is already considered one of the 100 'Worst Invasive Alien species in the Mediterranean' (Streftaris and Zenetos 2006). However, most of the invader species in the Mediterranean are native from tropical areas of the Indo-Pacific Ocean and Red Sea, which have entered through the Suez Canal. The most highlighting case is the blue swimming crab *Portunus segnis* (Forskål, 1775), another portunoid crab with similar invasive behavior than *C. sapidus* that recently showed a dramatic increase in abundance in the southern central Mediterranean (Gulf of Gabes, Tunisia, Crocetta et al. 2015). It inhabits sandy-muddy and seagrass bottoms of coastal and intertidal areas

96 and is native from the western Indian Ocean, Persian Gulf and Red Sea (Lai et al. 2010; Rabaoui
97 et al. 2015). It is considered one of the earliest alien crabs identified in the Mediterranean (Galil
98 2011) and its current distribution comprises the southern shores of the eastern and central
99 Mediterranean. *P. segnis* is an opportunistic benthic carnivore species with high competitive
100 ability and a great invasive potential given its efficient and fast reproductive and growth ability
101 (Pazooki et al. 2012; Safaie et al. 2013; Rabaoui et al. 2015) that would probably produce
102 important impacts in the structure of local communities. Considering the state of biodiversity
103 change that the Mediterranean Sea is experiencing in the last decades (Galil 2007; Katsanevakis
104 et al. 2014), it could be expected that this species would show a large process of geographical
105 expansion.

106 In brachyuran crabs, the presence of specific morphological features (e.g., spines, stronger
107 carapaces, efficient claws or adapted appendages) are common in many invasive species since
108 they influence positively their successful performance within native communities (Hartnoll 1971;
109 Lee 1995; Weis 2010; Daly et al. 2020). According to this, the main goal of the present study is
110 to determine the degree of morphological variability of the carapace shape of brachyuran crab
111 invaders in relation to native species from the local littoral community of Alfacs Bay, in the Ebro
112 Delta (NW Mediterranean). We selected this brachyuran community because it is well-known for
113 decades (Fusté 1988) and because is affected by two invader currently well-established species
114 (*C. sapidus* and *D. sayi*). Our initial hypothesis is that brachyuran invaders with uncommon
115 carapace shapes may possess certain ecological advantages in some essential life-history habits
116 (such as physiological adaptations to environment, reproduction efficiency, habitat use or
117 predator avoidance) as occurs in fishes with body shape (Azzurro et al. 2014; Smith et al. 2016;
118 Rojas-Vélez et al. 2019). Additionally, a hypothetical future scenario of entrance of a not yet
119 detected new invasive species in the study area, but already abundant in relatively nearby areas

(*P. segnis*) was also analyzed. Thus, the present study represents the first attempt to apply a morphological methodology to explore the potential invasive ability in marine decapod crustaceans.

Materials and methods

Study area and native crab community

The selected community of study was the brachyuran community inhabiting between 0-1 m in the Alfacs Bay, a semi-enclosed estuary of 3 m mean depth at the south side of the Ebro Delta region (NW Mediterranean), which is isolated from the open sea by a sandbar of 5 km long and 300 m wide (Fig. 1). This bay is mainly formed by sandy, muddy bottoms and seagrass meadows and receives freshwater from early spring to autumn through discharge channels that flow out from its northern extreme (Camp and Delgado 1987; Solé et al. 2009). Given its estuarine and brackish conditions, this area has been the object of several crab invasions during the last decades (Schubart et al. 2012; Castejón and Guerao 2013), this being the main reason why this location and faunistc assemblage were selected for the present study.

The specific composition of the native crab community was extracted from Fusté (1988), who studied monthly the decapod fauna from the bay during two years (march 1984-1986) using two different gears, traditionally used by the local artisanal fleet (trammel nets and 'rastell' bottom-towed dredges, Fusté 1988) over sandy and muddy bottoms of about 0-1 m depth. A total of 19 crab species belonging to 13 superfamilies were identified and considered as the native or base-line community (Table 1). Unfortunately, since then no additional similar samplings have been performed to determine the composition of the crab assemblage of this area. However, personal

144 communications with local fisheries administration and fishermen suggest that changes on
145 composition do not seem to be noticeable, and therefore it was considered the assemblage defined
146 by Fusté (1988) as representative of the current crab assemblage.
147
148 Carapace shape characterization and morphospace construction
149
150 The morphological description was performed using landmark-based geometric morphomterics.
151 The specimens used for the present study were obtained from the Biological Reference
152 Collections (CBR, http://cbr.icm.csic.es) of the Institute of Marine Sciences of Barcelona (ICM).
153 Due to the noticeable sexual and ontogenetic dimorphism that crabs present in their general body
154 shape (Rufino et al. 2004; Alencar et al. 2014), as well as for the higher availability of male
155 specimens for most of species, only adult males were analyzed in the present study in order to
156 avoid bias during the comparative shape analysis (Table 1). The initial intention was to include
157 the measurement of both carapace and other appendages (claws and pleopods) shape. However,
158 given the fragility of appendages and the state of conservation of the specimens, in many cases
159 they were incomplete and lacked of the claws, pleopods or both. Thus, in order to avoid bias
160 along the comparative morphometric process, we decided to discard both type of appendages
161 from the study and only describe the carapace shape. Firstly, each specimen was photographed in
162 dorsal view in standardized and scaled positions. The scheme of landmarks (fixed homologous
163 points) and semilandmarks (sliding or mobile non-homologous points), consisting in 10 different
164 points defining the general dorsal carapace shape (Fig. 2), was selected based on commonly used
165 configurations in previous morphometric studies with the aim of capturing the maximum of body
166 shape information (a detailed description of the meaning of landmarks and semilandmarks and
167 the used bibliography is provided in Supplementary material, Table S1). In order to avoid

168 duplication of equivalent landmarks in symmetrical structures (as is the case of the crabs
169 carapaces) that causes computation distortions during the analytical process, only one side of the
170 carapace was considered in the definition of landmarks (Rohlf and Slice 1990; Rufino et al. 2004,
171 2006).

172 The digitalization of landmarks and semilandmarks in all male specimens of each species was
173 performed using tpsDig v. 2.30 (Rohlf 2017a). Then, assuming that intraspecific variability is
174 lower than interspecific morphological variability, we obtained a consensus configuration for
175 each species that were used as the mean shape of each species in the remaining analyses. In order
176 to remove scale, orientation and size distortions not related with shape, a generalized Procrustes
177 analysis (GPA) was applied using tpsRelw v. 1.69 (Rohlf 2017b), which translates all
178 configurations to a common centroid position in the coordinate system, scales them to unit
179 centroid size and rotates them to minimise the distances between corresponding landmarks
180 (Adams et al. 2004; Zelditch et al. 2012). Initially, GPA coordinates are not expressed within an
181 Euclidean space shape. To project these Procrustes coordinates in a tangent space and test
182 whether shape variation is small enough to consider this new space a good image of the
183 Euclidean space (Rohlf 1999), we computed the correlation between the tangent and Procrustes
184 distances using tpsSmall v. 1.34 (Rohlf 2017c). The results of correlation (uncentered
185 correlation=0.999933, root mean square error=0.000501) confirmed that both spaces were nearly
186 identical for our data.

187 GPA also generates the uniform components of the shape variation for each analyzed object
188 (relative warps, RWs) by applying the thin-plate spline procedure, which creates a consensus
189 configuration by averaging the coordinates of all the landmarks. Each relative warp represents a
190 specific set of morphological characteristics, allowing to visualize the changes in shape between
191 species (Zelditch et al. 2012; Farré et al. 2016). The scores of the first two warps, which reached

192 more than 85% of the total morphological variation, were used to plot the morphological position
193 of each species in a two-dimensional graphic (morphospace) using PAST software v. 3.16
194 (Hammer et al. 2001).
195
196 Morphological variability analysis
197
198 The morphological dissimilarity within the morphospace of the community was computed from
199 methods based on the position of all species in the native morphospace and the distances between
200 them. Specifically, four variables were estimated: a) the convex hull, which was used to measure
201 the total dimension and shape of the assemblage morphospace and for further analyses. It is
202 defined as the smallest space enclosing all the individuals of a group (e.g, species of a community
203 or specimens of a species, Cornwell et al. 2006) and was computed in two different ways: at
204 specific level, in order to group by species and determine the intraspecific morphological
205 variability for each species within the morphospace, and at community level (obtained using the
206 consensus configuration of each species, see morphospace construction section) for the later
207 computation of Voronoi polygons. In both cases, it was built using Delone software v. 1.0.1.19;
208 b) the nearest-neighbour Euclidean distances (NND) between all the species within the
209 morphospace, which identify their 'closest' neighbours (Smith et al. 2016) and measure the
210 distances between them, and that were obtained using the package *spatstat* v. 1.59 (Baddeley et
211 al. 2015) in R (R Development Core Team 2017); c) the configuration and area of Voronoi
212 polygons (VOR), a tessellation method determined by distances among species in the space that
213 creates polygons enclosing the area belonging to each species (Du et al. 2012), which were
214 estimated using Image-Pro Plus, v. 5.0.0.39 software (Media Cybernetics, Inc.). Voronoi
215 polygons were created from the convex hull of the overall community morphospace obtained

from the consensus configuration of each species. The position within the community morphospace and the size of the Voronoi cells can be explanatory of the available 'niche opportunities' for species within a community (Azzurro et al. 2014; Smith et al. 2016); and finally d) the morphological disparity (MD), which measured the intraspecific morphological variation among species (Zelditch et al. 2012) and that was obtained with the package *geomorph* (Adams et al., 2020) in R (R Development Core Team 2017).

In order to characterize and assess the temporal evolution of the crab community morphospace until present time, as well as consider possible future scenarios of hypothetical new invasions, the morphospace representation and the estimation of all variables (convex hull, VOR, NND and MD) were computed at three different temporal moments: 1) considering the native crab community (NCC) described by Fusté (1988); 2) including the non-native but currently established species *D. sayi* and *C. sapidus* to the native community (current crab community, CCC); and 3) considering the hypothetical future entrance of *P. segnis* to the current crab community, thus imagining a potential future crab community (FCC). Assuming that the three scenarios are temporally consecutive, changes in the morphospace structures and dissimilarity measures were only compared (as shifts in magnitude) with respect to the previous time period.

Results

Morphospace configurations evidenced morphological variability along the crab communities (Fig. 3a-c). Species segregated along the *x* axis (RW1, 70.3% of total variance) based on the general carapace shape (total width and antero-posterior elongation). In the positive extreme were found species with antero-posteriorly elongated and laterally compressed carapaces, with shorter postero-lateral margins (*Macropodia* spp., *Parthenopoides massena*, *Corystes cassivelaunus*),

240 whereas in the negative extreme were located species with wider and antero-posteriorly shorter
241 carapaces as well as longer postero-lateral margins (*Goneplax rhomboides*, *C. sapidus*, *P.*
242 *segnis*). In contrast, throughout the *y* axis (RW2, 15.8% of total variation) species spread
243 according to the relation between the size of the anterior and posterior regions of the carapace.
244 The negative side was occupied by species with anterior and posterior regions of similar sizes (*C.*
245 *cassivelaunus*, *Atelecyclus rotundatus*, *Ilia nucleus*), while in the positive side were placed
246 species with anterior regions more developed than posterior regions (*C. sapidus*, *P. segnis*,
247 *Calappa granulata*, *Medorippe lanata*).

248 The morphospace and convex hull of the native community (NCC, Fig. 3a) was markedly
249 modified by the extremely outside location, oversteping the native limits, of *C. sapidus* (CCC,
250 Fig. 3b). Its particular carapace shape led the species to occupy a relatively large morphospace
251 portion away from the closest species, *Xantho poressa* (Table 1). Nevertheless, it located
252 relatively near from the core of hexagonal shapes, being one of the species that showed higher
253 intraspecific phenotypic variation (Table 1). By contrast, *D. sayi* showed a similar morphology to
254 some portunoid, grapsoid and xanthoid native species (*Liocarcinus* spp., *Necora puber*, *Carcinus*
255 *aestuarii, Brachynotus sexdentatus, X. poressa)* and thus located close to these native species
256 more centrally inside the convex hull of the morphospace, taking up less space and supplying
257 little morphological disparity in relation to closer species (Fig. 3b, Table 1).

258 Finally, in the future hypothetical scenario of new invasion in the current community (FCC),
259 the potential invader *P. segnis* presented a strong carapace shape similarity with *C. sapidus*
260 although not so extreme, locating inside the limits of the convex hull (Fig. 3c). The shape
261 resemblance with the currently established invader caused that presented low morphological
262 disparity (Table 1). However, its specific location allowed him to acquire a high VOR value (Fig

263 3c, Table 1), noticeably affecting to the occupied space by their surrounding species (*G.*
264 *rhomboides*, *Pilumnus spinifer*, *X. poressa* and especially *C. sapidus*).
265
266 **Discussion**
267
268 The present study analyzed the carapace morphological variability of a local brachyuran crab
269 assemblage of the Ebro delta, one of the largest estuarine zones of the Mediterranean Sea (Camp
270 and Delgado 1987; Guillén and Palanques 1997), affected by the invasion of several non-native
271 species. Our findings revealed a large interspecific diversity of carapace shapes for the native
272 species, including pyriform (*Macropodia* spp.), triangular (*P. massena*), circular (*I. nucleus*, *A.*
273 *rotundatus*), longitudinally ovate (*C. cassivelaunus*, *Maja crispata*), transversely ovate (*X.*
274 *poressa*), subovate (*C. granulata*), pentagonal (*M. lanata*), squarish (*G. rhomboides*) and a
275 majority of hexagonal (*Liocarcinus* spp., *B. sexdentatus*, *C. aestuarii*, *N. puber*) shapes
276 (following the terminology described by Ng 1998). Instead, the non-native species *D. sayi*
277 possesses a carapace with an intermediate shape between transversely ovate and common
278 hexagonal, whereas the invader *C. sapidus* presents an exclusive transversely hexagonal shape
279 characterized by extremely lengthened lateral spines. These phenotypes allowed them positioning
280 in different zones of the native morphospace: while *D. sayi* was noted within the morphospace
281 close to other native species, *C. sapidus* expanded the original limits of the morphospace and was
282 located in the periphery, distant from the nearest native species, occupying an 'empty' zone. This
283 dissimilarity in carapace shape showed by the newcomers coincides with ecological theories
284 supporting that successful invaders usually possess noticeable differences in many life-history
285 (including morphological) traits when compared with native species that allow them to reduce,
286 avoid or overcome competition with residents (Shea and Chesson 2002; Catford et al. 2009; Weis

287 2010; Parravicini et al. 2015). Carapace shape is recognized as an important trait in defining
288 ecological and biological habits of crab species related with reproductive, physiological, habitat
289 or behavioral strategies (Rufino et al. 2004; Silva et al. 2009; Olesen 2013). For instance, its
290 variability can be illustrative of strategies of sheltering and predator avoidance, determining
291 refuge preferences on rocky shores and salt marsh habitats (Idaszkin et al. 2013) or the burrowing
292 and burying ability of crabs in soft bottoms, since excavating mechanics, depth of concealment
293 and associated physiological adaptations are usually related with the carapace shape (Taylor and
294 Atkinson 1991; Bellwood 2002; Veas et al. 2014). Also the presence of spination over and
295 around the carapace margins is identified as an anti-predator adaptation (Davie et al. 2015; Daly
296 et al. 2020).

297 The real magnitude of success of crab invasive processes importantly depends on many other
298 factors beyond the carapace morphology of species related with, among others, the ecological,
299 biological and behavioural adaptability of non-native species (e.g., body size, agressivennes,
300 competition degree, larval lifespan and dispersion, physiological tolerance) and with the abiotic
301 and biotic environmental conditions (e.g., presence of predators, pathogens, interactions and
302 resistance with native species) of the receiving ecosystem (Catford et al 2009; Weis 2010;
303 Ricciardi et al 2013). Nevertheless, diverse studies suggest that non-native species locating in
304 peripheral positions within morphospaces, such as *C. sapidus*, tend to be highly successful or top
305 invaders (Azzurro et al. 2014; Smith et al. 2016; Rojas-Vélez et al. 2019; Tuset et al. 2020).
306 Considering the high invasive potential of *C. sapidus*, which has led the species to colonize
307 practically the entire Mediterranean basin (Galil et al. 2002; Streftaris and Zenetos 2006),
308 including the study area (López and Rodon 2018), it is reasonable to assume that its particular
309 carapace shape (completely disimilar to the remainig species) could also partially contribute,
310 among many other factors, to its successful performance for several reasons: a) the over-

development of the anterior part of the carapace (noted by the considerable expansion in *y* axis) entails a widening of the branchial region and antero-lateral margin. This would allow an enlargement of the gill chambers and surface, improving thus its respiratory efficiency (Millikin and Williams 1984; DeFur et al. 1988) and the capability to avoid desiccation by assuring more water conservation (Hopkins and Thurman 2010; Hampton et al. 2014); b) its large-sized stout, thick and flattened carapace characterized by strong spines along the antero-lateral margin finishing with especially lengthened lateral spines (Pyle and Cronin 1950; Millikin and Williams 1984) would act as a defensive adaptation against predators, even providing advantages over native competitors (Davis et al. 2004; Young et al. 2008); or c) this wider-shaped carapace would also contribute to the reproductive efficiency, providing females with a larger surface and volume for carrying and brooding fertilized eggs and favoring high fecundity rates (Van Engel 1958; Prager et al. 1990; Jivoff et al. 2007; Darnell et al. 2009). Instead, the intermediate positioning of *D. sayi* within the morphospace may indicate a higher ecological overlap, and thus direct competition, with the morphologically close native residents. Its carapace shape, between hexagonal and transversely ovate, can be considered a strategical advantage for occupying tridimensional complex habitats (Williams 1984; Micu et al. 2010, Cabiddu et al. 2020). In fact, the species is able to coexist with species typically from sandy and muddy bottoms (*Liocarcinus* spp., *B. sexdentatus*, *C. aestuarii*, Froglia and Speranza 1993; Mistri 2004) as well as with species over boulders and cobbles in fragmented rocky shores (*X. poressa*, Reuschel and Schubart 2007; Spivak et al. 2010). This high habitat adaptability of the species can help to understand its colonization along almost the whole Mediterranean Sea (Froglia and Speranza 1993; Mizzan 1995; Micu et al. 2010; Schubart et al. 2012; Thessalou-Legaki et al. 2012; Ulman et al. 2017). However, when habitat complexity is more reduced, given its small size and that it rarely buries, this species can be preyed by larger-sized species such as *C. aestuarii* (Gehrels et

335 al. 2016). This higher vulnerability to predation, together with the interspecific competition that
336 would have to confront with morphologically-close species, would support that the species,
337 despite its expansive and establishment ability, show a lower invasive potential than *C. sapidus*.
338 Following the same premises, results suggest that *P. segnis* might also be a top invader since its
339 morphological similarity in carapace shape with *C. sapidus* provides it with a similar ecological
340 and biological performance (Safaie et al. 2013; Crocetta et al. 2015; Rabaoui et al. 2015). Here
341 the interest would lie in assessing the result of its probable direct competition with the closer
342 residents especially with *C. sapidus*, since it would probably exercise resistance limiting the
343 entrance of the newcomer, as occurred in experiments between *C. sapidus* and *Carcinus maenas*
344 (DeRivera et al. 2005). In any case, given that the direct competence would affect all species, it
345 would be plausible that *P. segnis* could stand competence and coexist with the previously
346 established species (MacDonald et al. 2007), and therefore become a invader with a high
347 probability to establish within the community (Katsanevakis et al. 2014).

348 Brachyuran crabs are the most numerous and diverse group of decapods occupying a wide
349 spectrum of ecological niches and lifestyles (Davie et al. 2015) and their particular crab-shape
350 and high phenotypic variability have always been considered as fundamental to explain their
351 great diversity and evolutionary success (Števčić 1971; Förster 1985; Scholtz 2014). The local
352 crab community analyzed in the present study represents a good illustrative example of this
353 morphological diversification, showing a broad range of different carapace shapes and some
354 redundancy of hexagonal shapes characteristic from portunoid (*Liocarcinus* spp, *C. aestuarii*, *N.*
355 *puber*), grapsoid (*B. sexdentatus*) or pilumnoid (*P. spinifer*) species. Considering that under
356 particular environmental conditions the selection towards certain specialized phenotypes that
357 maximize the ecological efficiency of species is common (environmental filtering hypothesis,
358 Zobel 1997), this carapace shape (similar to that of invader species) could be interpreted as an

359 optimal trait for the specific studied area. Therefore, the entrance of newcomers (including the
360 future hypothetical case) would probably entail negative effects, at greater or lesser extent, to
361 these native species with similar morphologies by increasing competence and reducing their
362 ecological niche. On the other hand, native species with higher phenotypic variability (e.g.,
363 *Liocarcinus depurator* or *X. poressa*) could exhibit a higher resilience to the perturbations caused
364 by the invasive processes (Allen and Holling 2010; Berthon 2015). In any case, the occurrence of
365 invader species will produce certain degree of alteration in the structure and functioning of the
366 native assemblage. However, it is difficult to identify and quantify the real specific impacts that
367 invaders would cause to native residents considering only the carapace shape as niche trait and
368 with no abundance data from the community. A more complete definition of the ecological niche
369 of the community would be necessary to unravel the real effects of invaders on the residents,
370 which would require considering other morphological features (e.g., body size, size and shape of
371 claws or presence of transformed appendages such as flattened paddle-like swimming pleopods
372 and pereiopods) essential by habits such as with foraging, locomotion, defensive, aggressiveness,
373 competitive and mating strategies of crabs (Hartnoll 1971; Vermeij1977; Hines 1982; Lee 1995;
374 Sneddon et al. 1997; Mariappan et al. 2000). Unfortunately, the measuring of some of these
375 structures (claws and pleopods) could not be included in the present study due to methodological
376 difficulties, entailing evident limitations for the ecological interpretations of the study, and thus
377 are effusively encouraged to be included in further studies.

378 In conclusion, the present study represents a first attempt to evaluate the potential effects of
379 the entrance of marine crustacean alien species (brachyuran decapods) within native assemblages
380 using a methodological approach based on the analysis of external morphology of species,
381 currently only applied in fishes both for body shape (Azzurro et al. 2014; Smith et al. 2016;
382 Rojas-Vélez et al. 2019) or for sensory structures such as otoliths (Tuset et al. 2020). The study

was performed in a local specific community, including a relatively small sampling size and from a very enclosed environment in terms of spatial and temporal coverage, which entails evident limitations. Further studies at larger spatial and temporal scales, including larger sets of species that encompass higher ecological and functional variability, should be performed in order to more robustly contrast the efficacy of this model in decapod crustaceans. Besides, a more complete morphological description including other anatomical structures of ecological interest such as claws and other appendages (Hartnoll 1971; Vermeij 1977; Lee 1995, Davie et al. 2015), would be necessary for a more deeply assessment of the ecological conclusions from the morphospace results. However, results based on carapace shape, a morphological trait also strongly related with the ecological performance of species, suggest that invaders that present differential morphological traits can possess ecological advantages that would increase their likelihood to establish themselves since they avoid competition with similar residents. The study demonstrates that this straightforward morphometric protocol can be employed as an alternative effective tool to preliminary assess the effects of invasive processes of species from different taxa and phyla as well as when ecological data of invader species is scarce. Notwithstanding, it should be complemented by studies incorporating accurate quantitative ecological information of the invasive events to extract more robust conclusions about the evolution of invasive species within native communities.

Author's contribution

All authors contributed to the study conception and design. MF and PA selected and prepared samples for the study; MF and AL performed the morphological analyses; VMT performed some of the statistical analyses; all authors interpreted data. MF and VMT wrote the first draft of the

406 manuscript. All authors commented on previous versions of the manuscript and approved the
407 final manuscript.
408
409 **Funding**
410 The present study is included and financed within the framework of the project CLIFISH
411 (CTM2015-66400-C3-3-R, MINECO/FEDER), funded by the Spanish Ministry of Economy and
412 Competitiveness and by the European Regional Development Fund (ERDF).
413
414 **Compliance with Ethical Standards**
415 **Conflict of interest**
416 The authors declare that they have no conflict of interest.
417 **Ethical approval**
418 All the research was performed following the general guidelines for the ethical use of animals.
419
420 **References**
421
422 Adams DC, Collyer M, Kaliontzopoulou A (2020) Geometric Morphometric Analyses of 2D/3D
423 Landmark Data, CRAN. Available at https://cran.r-project.org/web/ packages/geomorph/
424 geomorph.pdf
425 Adams DC, Rohlf FJ, Slice DE (2004) Geometric morphometrics: ten years of progress
426 following the "revolution." Ital J Zool 71:5–16
427 Alencar CERD, Lima-Filho PA, Molina WF, Freire FAM (2014) Sexual shape dimorphism of the
428 mangrove crab *Ucides cordatus* (Linnaeus, 1763) (Decapoda, Ucididae) accessed through
429 geometric morphometric. Sci World J 2014:206168. doi: 10.1155/2014/206168
430 Allen CR, Holling CS (2010) Novelty, Adaptive Capacity, and Resilience. Ecol Soc 15:24
431 Azzurro E, Tuset VM, Lombarte A, Maynou F, Simberloff D, Rodríguez-Pérez A, Solé RV

(2014) External morphology explains the success of biological invasions. Ecol Lett 17:1455–1463. doi: 10.1111/ele.12351

Baddeley A, Rubak E, Turner R (2015) Spatial Point Patterns: Methodology and Applications with R. London: Chapman and Hall/CRC Press, 2015. http://www.crcpress.com/Spatial-Point-Patterns-Methodology-and-Applications-with-R/Baddeley-Rubak-Turner/9781482210200/

Bellwood O (2002) The occurrence, mechanics and significance of burying behaviour in crabs (Crustacea: Brachyura). J Nat Hist 36:1223–1238. doi: 10.1080/00222930110048891

Berthon K (2015) How do native species respond to invaders? Mechanistic and trait-based perspectives. Biol Invasions 17:2199–2211. doi: 10.1007/s10530-015-0874-7

Cabiddu S, Addis P, Palmas F, Pusceddu A (2020) First record of *Dyspanopeus sayi* (Smith, 1869) (decapoda: Brachyura: Panopeidae) in a Sardinian coastal lagoon (western mediterranean, Italy). BioInvasions Rec 9:74–82. doi: 10.3391/bir.2020.9.1.10

Camp J, Delgado M (1987) Hidrografía de las bahías del Delta del Ebro. Investig Pesq 51:351–359

Castejón D, Guerao G (2013) A new record of the American blue crab, *Callinectes sapidus* Rathbun, 1896 (Decapoda: Brachyura: Portunidae), from the Mediterranean coast of the Iberian Peninsula. Bioinvasions Rec 2:141–143. doi: 10.3391/bir.2013.2.2.08

Catford J, Jansson R, Nilsson C (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Divers Distrib 15:22–40. doi: 10.1111/j.1472-4642.2008.00521.x

Cornwell W, Schwilk D, Ackerly D (2006) A trait-based test for habitat filtering: convex hull volume. Ecology 87:1465–1471.

Crocetta F, Agius D, Balistreri P, Bariche M, Bayhan Y, Çakir M, Ciriaco S, Corsini-Foka M, Deidun A, El Zrelli R, Ergüden D, Evans J, Ghelia M, Giavasi M, Kleitou P, Kondylatos G, Lipej L, Mifsud C, Özvarol Y, Pagano A, Portelli P, Poursanidis D, Rabaoui L, Schembri P, Taskin E, Tiralongo F, Zenetos A (2015) New Mediterranean Biodiversity Records (October 2015). Mediterr Mar Sci 16:682–702

Cury PM, Shin YJ, Planque B, Durant JM, Fromentin JM, Kramer-Schadt S, Stenseth NC, Travers M, Grimm V (2008) Ecosystem oceanography for global change in fisheries. Trends Ecol Evol 23:338–346. doi: 10.1016/j.tree.2008.02.005

463 Daly BJ, Eckert GL, Long WC (2020) Moulding the ideal crab: implications of phenotypic
464 plasticity for crustacean stock enhancement. ICES J Mar Sci. doi: 10.1093/icesjms/fsaa043
465 Darnell MZ, Rittschof D, Darnell KM, McDowell RE (2009) Lifetime reproductive potential of
466 female blue crabs *Callinectes sapidus* in North Carolina, USA. Mar Ecol Prog Ser 394:153–
467 163. doi: 10.3354/meps08295
468 Davie PJF, Guinot D, Ng PKL (2015) Anatomy and Functional Morphology of Brachyura. In:
469 Castro P, Davie PJF, Guinot D, Schram FR, von Vaupel Klein, JC (eds) Treatise on Zoology
470 – Anatomy, Taxonomy, Biology. The Crustacea. Volume 9C. Decapoda: Brachyura (Part 1).
471 pp 11–163. https://doi.org/10.1163/9789004190832_004
472 Davis JLD, Young-Williams AC, Aguilar R, Carswell BL, Goodison, MR, Hines, AH, Kramer,
473 MA, Zohar Y, Zmora O (2004) Differences between Hatchery-Raised and Wild Blue Crabs:
474 Implications for Stock Enhancement Potential. Trans Am Fish Soc 133:1–14. doi:
475 10.1577/t03-004
476 DeFur PL, Pease A, Siebelink A, Elfers S (1988) Respiratory responses of blue crabs, *Callinectes*
477 *sapidus*, to emersion. Comp Biochem Physiol Part A Physiol 89:97–101
478 DeRivera CE, Ruiz GM, Hines AH, Jivoff P (2005) Biotic Resistance to Invasion : Native
479 Predator Limits Abundance and Distribution of an Introduced Crab. Ecology 86:3364–3376
480 Du F, Xu X, Zhang XC, Sui Y, Shao M, Hu L, Shan L (2012) The relationships between
481 aboveground biomass and Voronoi area of coexisting species in an old-field community.
482 Polish J Ecol 60:479–489
483 Farré M, Tuset VM, Cartes JE, Massutí E, Lombarte A (2016) Depth-related trends in
484 morphological and functional diversity of demersal fish assemblages in the western
485 Mediterranean Sea. Prog Oceanogr 147:22–37. doi: 10.1016/j.pocean.2016.07.006
486 Ficetola GF, Thuiller W, Miaud C (2007) Prediction and validation of the potential global
487 distribution of a problematic alien invasive species - The American bullfrog. Divers Distrib
488 13:476–485. doi: 10.1111/j.1472-4642.2007.00377.x
489 Förster R (1985) Evolutionary trends and ecology of Mesozoic decapod crustaceans. Trans R Soc
490 Edinburgh 76:299–304. doi: 10.1017/S0263593300010518
491 Froglia C, Speranza S (1993) First record of *Dyspanopeus sayi* (Smith, 1869) in the
492 Mediterranean Sea (Crustacea: Decapoda: Xanthidae). Quad Ist Ric Pesca Marittima 5:163–
493 166

494 Fusté X (1988) Crustaceos decapodos de la Bahia de els Alfacs (Delta del Ebro). Inv. Pesq.
495 52:617–623
496 Galil B (2007) Loss or gain? Invasive aliens and biodiversity in the Mediterranean Sea. Mar
497 pollut bull 55(7-9):314–322. doi: https://doi.org/10.1016/j.marpolbul.2006.11.008
498 Galil B (2011) The Alien Crustaceans in the Mediterranean Sea: An Historical Review. In: Galil
499 B, Clark P, Carlton J (eds) In the Wrong Place - Alien Marine Crustaceans: Distribution,
500 Biology and Impacts. Invading Nature - Springer Series in Invasion Ecology, 6, pp 377–402
501 Galil B, Froglia C, Noël P (2002) CIESM atlas of exotic species in the Mediterranean, Volumen
502 2. Crustaceans: decapods and stomatopods. CIESM Publishers, Monaco
503 Gehrels H, Knysh KM, Boudreau M, Thériault MH, Courtenay SC, Cox R, Quijón PA (2016)
504 Hide and seek: habitat-mediated interactions between European green crabs and native mud
505 crabs in Atlantic Canada. Mar Biol 163:152. doi: 10.1007/s00227-016-2927-6
506 González JA, Triay-Portella R, Escribano A, Cuesta JA (2017) Northernmost record of the
507 pantropical portunid crab *Cronius ruber* in the eastern Atlantic (Canary Islands): natural
508 range extension or human-mediated introduction? Sci Mar 81:81–89. doi:
509 10.3989/scimar.04551.17b
510 Grosholz E (2002) Ecological and evolutionary consequences of coastal invasions. Trends Ecol
511 Evol 17:22–27. doi: 10.1016/S0169-5347(01)02358-8
512 Guillén J, Palanques A (1997) A historical perspective of the morphological evolution in the
513 lower Ebro river. Environ Geol 30:174–180. doi: 10.1007/s002540050144
514 Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS,
515 Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER,
516 Spalding M, Steneck R, Watson R (2008) A Global Map of Human Impact on Marine
517 Ecosystems. Science 319:948–952. doi: 10.1126/science.1149345.
518 Hammer Ø, Harper DA, Ryan PD (2001) PAST: Paleontological Statistics software package for
519 education and data analysis. Paleontol Electron 4:9 pp
520 Hampton KR, Hopkins MJ, Mcnamara JC, Thurman CL (2014) Intraspecific variation in
521 carapace morphology among fiddler crabs (Genus *Uca*) from the Atlantic coast of Brazil.
522 Aquat Biol 20:53–67. doi: 10.3354/ab00545
523 Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF,
524 Tomaner L, Williams SL (2006) The impacts of climate change in coastal marine systems.

525 Ecol Lett 9:228–241. doi: 10.1111/j.1461-0248.2005.00871.x
526 Harris LG, Tyrrell MC (2001) Changing community states in the Gulf of Maine: Synergism
527 between invaders, overfishing and climate change. Biol Invasions 3:9–21. doi:
528 10.1023/A:1011487219735
529 Hartnoll RG (1971) The occurrence, methods and significance of swimming in the Brachyura.
530 Anim Behav 19:34–50. doi: 10.1016/S0003-3472(71)80132-X
531 Hidalgo M, Rouyer T, Molinero JC, Massutí E, Moranta J, Guijarro B, Stenseth NC (2011)
532 Synergistic effects of fishing-induced demographic changes and climate variation on fish
533 population dynamics. Mar Ecol Prog Ser 426:1–12. doi: 10.3354/meps09077
534 Hill J, Fowler DL, Van den Avyle MJ (1989) Species Profiles. Life Histories and Environmental
535 Requirements of Coastal Fishes and Invertebrates (Mid-Atlantic). Blue Crab. U.S. Army
536 Corps of Engineers, Vicksburg
537 Hines AH (1982) Coexistence in a Kelp Forest: Size, Population Dynamics, and Resource
538 Partitioning in a Guild of Spider Crabs (Brachyura, Majidae). Ecol Monogr 52:179–198.
539 doi: 10.2307/1942610
540 Hoegh-Guldberg O, Bruno JF (2010) The Impact of Climate Change on the World's Marine
541 Ecosystems. Science 328:1523–1528. doi: 10.1080/00330124.2015.1124788
542 Hopkins MJ, Thurman CL (2010) The geographic structure of morphological variation in eight
543 species of fiddler crabs (Ocypodidae: Genus *Uca*) from the eastern United States and
544 Mexico. Biol J Linn Soc 100:248–270. doi: 10.1111/j.1095-8312.2010.01402.x
545 Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-
546 Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nyström M, Palumbi SR,
547 Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the
548 resilience of coral reefs. Science 301:929–934. doi: 10.1126/science.1085046
549 Idaszkin YL, Márquez F, Nocera AC (2013) Habitat-specific shape variation in the carapace of
550 the crab *Cyrtograpsus angulatus*. J Zool 290:117–126. doi: 10.1111/jzo.12019
551 Jivoff P, Hines AH, Quackenbush LS (2007) Reproduction biology and embryonic development.
552 In: Kennedy VS, Cronin LE (eds) The Blue Crab: *Callinectes sapidus*. Maryland Sea Grant
553 College, College Park, Maryland, pp 255–298
554 Karatayev AY, Burlakova LE, Padilla DK, Mastitsky SE, Olenin S (2009) Invaders are not a
555 random selection of species. Biol Invasions 11:2009–2019. doi: 10.1007/s10530-009-9498-0

556 Katsanevakis S, Wallentinus I, Leppäkoski E, Çinar ME, Oztürk B, Grabowski M, Golani D,
557 Cardoso AC (2014) Impacts of Invasive Alien Marine Species on Ecosystem Services and
558 Biodiversity: a pan-European Review. Aquat Invasions 9:391–423. doi:
559 10.3391/ai.2014.9.4.01
560 Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol
561 16:199–204
562 Lai JCY, Ng PKL, Davie PJF (2010) A revision of the *Portunus pelagicus* (Linnaeus, 1758)
563 species complex (Crustacea: Brachyura: Portunidae), with the recognition of four species.
564 Raffles Bull Zool 58:199–237
565 Lee SY (1995) Cheliped size and structure: the evolution of a multi-functional decapod organ. J
566 Exp Mar Bio Ecol 193:161–176. doi: 10.1016/0022-0981(95)00116-6
567 Lejeusne C, Latchere O, Petit N, Rico C, Green AJ (2014) Do invaders always perform better?
568 Comparing the response of native and invasive shrimps to temperature and salinity gradients
569 in south-west Spain. Estuar Coast Shelf Sci 136:102–111. doi: 10.1016/j.ecss.2013.11.014
570 López V, Rodon J (2018) Diagnosi i situació actual del Cranc Blau (*Callinectes sapidus*) al delta
571 de l' Ebre. Informe Tècnic Servei de Recursos Marins. Direcció General de Pesca i Afers
572 Marítims, Generalitat de Catalunya, 86 pp
573 MacDonald JA, Roudez R, Glover T, Weis JS (2007) The invasive green crab and Japanese shore
574 crab: Behavioral interactions with a native crab species, the blue crab. Biol Invasions 9:837–
575 848. doi: 10.1007/s10530-006-9085-6
576 Mancinelli G, Carrozzo L, Costantini ML, Rossi L, Marini G, Pinna M (2013) Occurrence of the
577 Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 in two Mediterranean coastal habitats:
578 Temporary visitor or permanent resident? Estuar Coast Shelf Sci 135:46–56. doi:
579 10.1016/j.ecss.2013.06.008
580 Mariappan P, Balasundaram C, Schmitz B (2000) Decapod crustacean chelipeds: An overview. J
581 Biosci 25:301–313. doi: 10.1007/BF02703939
582 Micu D, Niţă V, Todorova V (2010) First record of Say's mud crab *Dyspanopeus sayi*
583 (Brachyura: Xanthoidea: Panopeidae) from the Black Sea. Mar Biodivers Rec 3:1–6. doi:
584 10.1017/S1755267210000308
585 Millikin MR, Williams AB (1984) Synopsis of biological data on blue crab, *Callinectes sapidus*
586 Rathbun. FAO Fish Synopsis

587 Mistri M (2004) Predatory behavior and preference of a successful invader, the mud crab
588 *Dyspanopeus sayi* (Panopeidae), on its bivalve prey. J Exp Mar Bio Ecol 312:385–398. doi:
589 10.1016/j.jembe.2004.07.012
590 Mizzan L (1995) Notes on presence and diffusion of *Dyspanopeus sayi* (Smith, 1869) (Crustacea,
591 Decapoda, Xanthidae) in the Venetian Lagoon. Boll Mus Civ St Nat Venezia 44:121–129
592 Nehring S (2011) Invasion History and Success of the American Blue Crab *Callinectes sapidus*
593 in European and Adjacent Waters. In: Galil B, Clark P, Carlton J (eds) In the Wrong Place -
594 Alien Marine Crustaceans: Distribution, Biology and Impacts. Invading Nature - Springer
595 Series in Invasion Ecology, 6: 607–624
596 Ng PKL (1998) Crabs. In: Carpenter KE, Niem VH (eds) FAO Species Identification Guide for
597 Fishery Purposes. The Living Marine Resources of the Western Central Pacific. Volume 2:
598 Cephalopods, crustaceans, holothurians and sharks. pp 1045–1055
599 Nizinski MS (2003) Annotated checklist of decapod crustaceans of Atlantic coastal and
600 continental shelf waters of the United States. Proc Biol Soc Washingt 116:96–157
601 Olesen J (2013) The crustacean carapace: morphology, function, development, and phylogenetic
602 history. In: Watling L, Thiel M (eds) Functional Morphology and Diversity of Crustaceans.
603 The Natural History of the Crustacea. Volume 1. Oxford University Press, Oxford, pp 103–
604 139
605 Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across
606 natural systems. Nature 421:37–42. doi: 10.1038/nature01286
607 Parravicini V, Azzurro E, Kulbicki M, Belmaker J (2015) Niche shift can impair the ability to
608 predict invasion risk in the marine realm: An illustration using Mediterranean fish invaders.
609 Ecol Lett 18:246–253. doi: 10.1111/ele.12401
610 Pazooki J, Hosseini M, Zadeh AV (2012) The dietary compositions of the blue swimming crab,
611 *Portunus segnis* (Forskal, 1775) from Persian Gulf, South Iran. World Appl Sci J 20:416–
612 422. doi: 10.5829/idosi.wasj.2012.20.03.1969
613 Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate Change and Distribution Shifts in
614 Marine Fishes. Science 308:1912-1915. doi: 10.1126/science.1111322
615 Philippart CJM, Anadón R, Danovaro R, Dippner JW, Drinkwater KF, Hawkins SJ, Oguz T,
616 O'Sullivan G, Reid PC (2011) Impacts of climate change on European marine ecosystems:
617 Observations, expectations and indicators. J Exp Mar Bio Ecol 400:52–69. doi:

618 10.1016/j.jembe.2011.02.023
619 Pörtner HO, Farrell P (2008) Physiology and Climate Change. Science 322:690–692. doi:
620 10.1007/s10502-012-9180-7
621 Prager MH, McConaugha JR, Jones CM, Geer PJ (1990) Fecundity of blue crab, *Callinectes*
622 *sapidus*, in Chesapeake Bay: biological, statistical and management considerations. Bull
623 Mar Sci 46:170–179
624 Pyle R, Cronin E (1950) The general anatomy of the blue crab *Callinectes sapidus* Rathbun.
625 Chesapeake Biological Laboratory Publications 87, 40 pp
626 R Development Core Team (2017) R: A language and environment for statistical computing. R
627 Foundation for Statistical Computing, Vienna, Austria
628 Rabaoui L, Arculeo M, Mansour L, Tlig-Zouari S (2015) Occurrence of the lessepsian species
629 *Portunus segnis* (Crustacea: Decapoda) in the Gulf of Gabes (Tunisia): First record and new
630 information on its biology and ecology. Cah Biol Mar 56:169–175
631 Reuschel S, Schubart CD (2007) Contrasting genetic diversity with phenotypic diversity in
632 coloration and size in *Xantho poressa* (Brachyura: Xanthidae), with new results on its
633 ecology. Mar Ecol 28:296–305. doi: 10.1111/j.1439-0485.2006.00139.x
634 Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the
635 ecological impacts of nonnative species. Ecol Monogr 83:263–282. doi: 10.1890/13-0183.1
636 Rohlf FJ (1999) Shape statistics: Procrustes superimpositions and tangent spaces. J Classif
637 16:197–223
638 Rohlf FJ (2017a) TPS Dig Version 2.30 and TPS Relative Warps Software. State University of
639 New York at Stony Brook
640 Rohlf FJ (2017b) TPS Relw Version 1.69 and TPS RelativeWarps Software. State University of
641 New York at Stony Brook
642 Rohlf FJ (2017c) TpsSmall Version 1.34 and TPS Relative Warps Software. State University of
643 New York at Stony Brook
644 Rohlf FJ, Slice D (1990) Extensions of the Procrustes method for the optimal superimposition of
645 landmarks. Syst Biol 39:40–59
646 Rojas-Vélez S, Tavera J, Acero A (2019) Unraveling lionfish invasion: Is *Pterois volitans* truly a
647 morphologically novel predator in the Caribbean? Biol Invasions 21:1921–1931. doi:
648 10.1007/s10530-019-01946-6

649 Rufino MM, Abelló P, Jones DA (2004) Male and female carapace shape differences in
650 *Liocarcinus depurator* (Decapoda, Brachyura): An application of geometric morphometric
651 analysis to crustaceans. Ital. J. Zool. 71:79–83
652 Rufino MM, Abelló P, Yule AB (2006) Geographic and gender shape differences in the carapace
653 of *Liocarcinus depurator* (Brachyura: Portunidae) using geometric morphometrics and the
654 influence of a digitizing method. J Zool 269:458–465. doi: 10.1111/j.1469-
655 7998.2006.00086.x
656 Safaie M, Pazooki J, Kiabi B, Shokri MR (2013) Reproductive biology of blue swimming crab,
657 *Portunus segnis* (Forskal, 1775) in coastal waters of Persian Gulf and Oman Sea, Iran. Iran J
658 Fish Sci 12:430–444
659 Scholtz G (2014) Evolution of crabs - history and deconstruction of a prime example of
660 convergence. Contrib to Zool 83:87–105
661 Schubart CD, Guerao G, Abelló P (2012) First record and evidence of an established population
662 of the North American mud crab *Dyspanopeus sayi* (Brachyura: Heterotremata: Panopeidae)
663 in the western Mediterranean. Sci Mar 76:79–85. doi: 10.3989/scimar.03361.16A
664 Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions.
665 Trends Ecol Evol 17:170–176. doi: 10.1016/s0169-5347(02)02495-3
666 Silva IC, Hawkins SJ, Paula J (2009) A comparison of population differentiation in two shore
667 crab species with contrasting distribution along the Portuguese coast, using two
668 morphological methodologies. Mar Freshw Res 60:833–844
669 Simberloff D, Martin JL, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B,
670 García-Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of
671 biological invasions: What's what and the way forward. Trends Ecol Evol 28:58–66. doi:
672 10.1016/j.tree.2012.07.013
673 Smith SM, Fox RJ, Donelson JM, Head ML, Booth DJ (2016) Predicting range-shift success
674 potential for tropical marine fishes using external morphology. Biol Lett 12: 20160505. doi:
675 10.1098/rsbl.2016.0505
676 Sneddon LU, Huntingford FA, Taylor AC (1997) Weapon size versus body size as a predictor of
677 winning in fights between shore crabs, *Carcinus maenas* (L.). Behav Ecol Sociobiol
678 41:237–242
679 Solé J, Turiel A, Estrada M, Llebot C, Blasco D, Camp J, Delgado M, Fernández-Tejedor M,

680 Diogène J (2009) Climatic forcing on hydrography of a Mediterranean bay (Alfacs Bay).
681 Cont Shelf Res 29:1786–1800. doi: 10.1016/j.csr.2009.04.012
682 Spivak ED, Arévalo E, Cuesta JA, González-Gordillo JI (2010) Population structure and
683 reproductive biology of the stone crab *Xantho poressa* (Crustacea: Decapoda: Xanthidae) in
684 the 'Corrales de Rota' (south-western Spain), a human-modified intertidal fishing area. J
685 Mar Biol Assoc United Kingdom 90:323–334. doi: 10.1017/S0025315409990592
686 Stachowicz JJ, Terwin JR, Whitlatch RB, Osman RW (2002) Linking climate change and
687 biological invasions: Ocean warming facilitates nonindigenous species invasions. Proc Natl
688 Acad Sci 99:15497–15500. doi: 10.1073/pnas.242437499
689 Števčić Z (1971) The main features of brachyuran evolution. Syst Zool 20:331–340. doi:
690 10.2307/2412345
691 Streftaris N, Zenetos A (2006) Alien marine species in the Mediterranean - the 100 "worst
692 invasives" and their impact. Mediterr Mar Sci 7:87–118. doi: 10.12681/mms.180
693 Taylor AC, Atkinson RJA (1991) Respiratory adaptations of aquatic decapod crustaceans and
694 fish to a burrowing mode of life. In: Woakes AJ, Grieshaber MK, Bridges CR (eds)
695 Physiological Strategies for Gas Exchange and Metabolism: Society of Experimental
696 Biology, Seminar Series, Vol. 41. Cambridge University Press, Cambridge, UK, pp 211–234
697 Thessalou-Legaki M, Aydogan O, Bekas P, Bilge G, Boyaci YO, Brunelli E, Circosta V,
698 Crocetta F, Durucan F, Erdem M, Ergolavou A, Filiz H, Fois F, Gouva E, Kapiris K,
699 Katsanevakis S, Kljajić Z, Konstantinidis E, Konstantinou G, Koutsogiannopoulos D,
700 Lamon S, Mačić V, Mazzette R, Meloni D, Mureddu A, Paschos I, Perdikaris C, Piras F,
701 Poursanidis D, Ramos-Esplá AA, Rosso A, Sordino P, Sperone E, Sterioti A, Taşkin E,
702 Toscano F, Tripepi S, Tsiakkiros L, Zenetos A (2012) New Mediterranean Biodiversity
703 Records (December 2012). Mediterr Mar Sci 13:312–327
704 Tuset VM, Lombarte A, Bariche M, Maynou F, Azzurro E (2020) Otolith morphological
705 divergences of successful Lessepsian fishes on the Mediterranean coastal waters. Estuar
706 Coast Shelf Sci 236:106631. doi: 10.1016/j.ecss.2020.106631
707 Ulman A, Ferrario J, Occhipinti-Ambrogi A, Arvanitidis C, Bandi A, Bertolino M, Bogi C,
708 Chatzigeorgiou G,Çiçek BA, Deidun A, Ramos-Esplá A, Koçak C, Lorenti M, Martinez-
709 Laiz G, Merlo G, Princisgh E, Scribano G, Marchini A (2017) A massive update of non-
710 indigenous species records in Mediterranean marinas. PeerJ 5: e3954. doi:

711 10.7717/peerj.3954
712 Van Engel W (1958) The blue crab and its fishery in Chesapeake bay. Part 1. Reproduction, early
713 development, growth and migration. Commer Fish Rev 20:6–17
714 Veas R, Hernández-Miranda E, Quiñones RA (2014) Body shape and burial behavior of the sand
715 crab *Emerita analoga* (Stimpson 1857) in a reflective to intermediate morphodynamic range
716 of sandy beaches. Mar Biol 161:2345–2357. doi: 10.1007/s00227-014-2510-y
717 Vermeij GJ (1977) Patterns in Crab Claw Size : The Geography of Crushing. Syst Zool 26:138–
718 151
719 Walther GR, Roques A, Hulme PE, Sykes MT, Pyšek P, Kühn I, Zobel M, Bacher S, Botta-Dukát
720 Z, Bugmann H, Czúcz B, Dauber J, Hickler T, Jarošík V, Kenis M, Klotz S, Minchin D,
721 Moora M, Nentwig W, Ott J, Panov VE, Reineking B, Robinet C, Semenchenko V, Solarz
722 W, Thuiller W, Vilà M, Vohland K, Settele J (2009) Alien species in a warmer world: risks
723 and opportunities. Trends Ecol Evol 24:686–693. doi: 10.1016/j.tree.2009.06.008
724 Weis JS (2010) The role of behavior in the success of invasive crustaceans. Mar Freshw Behav
725 Physiol 43:83–98. doi: 10.1080/10236244.2010.480838
726 Wernberg T, Smale DA, Tuya F, Thomsen MS, Langlois TJ, De Bettignies T, Bennett S,
727 Rousseaux CS (2013) An extreme climatic event alters marine ecosystem structure in a
728 global biodiversity hotspot. Nat Clim Chang 3:78–82. doi: 10.1038/nclimate1627
729 Williams AB (1984) Shrimps, Lobsters, and Crabs of the Atlantic Coast of the Eastern United
730 States, Maine to Florida. Smithsonian Institution Press, Washington DC
731 Young AC, Johnson EG, Davis JLD, Hines AH, Zmora O, Zohar Y (2008) Do Hatchery-Reared
732 Blue Crabs Differ from Wild Crabs, and Does it Matter? Rev Fish Sci 16:254–261. doi:
733 10.1080/10641260701684122
734 Zelditch ML, Swiderski D, Sheets H (2012) Geometric morphometrics for biologists: a primer.
735 Elsevier Academic Press
736 Zobel M (1997) The relative role of species pools in determining plant species richness: An
737 alternative explanation of species coexistence? Trends Ecol Evol 12:266–269. doi:
738 10.1016/S0169-5347(97)01096-3
739
740
741

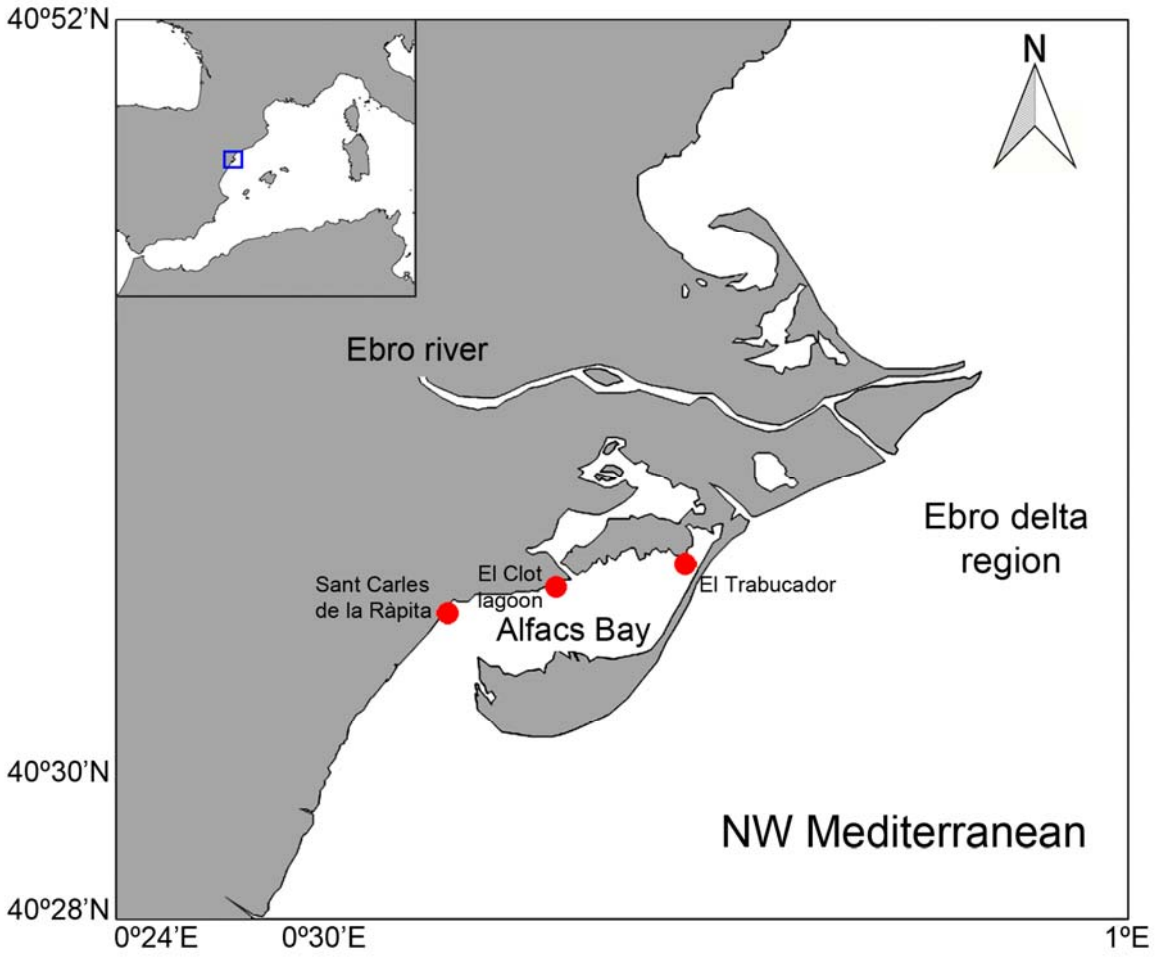


Fig. 1 Map of the study area (Alfacs bay, Ebro Delta region, NW Mediterranean) and specific areas where non-native species were collected. Adapted from Schubart et al. 2012

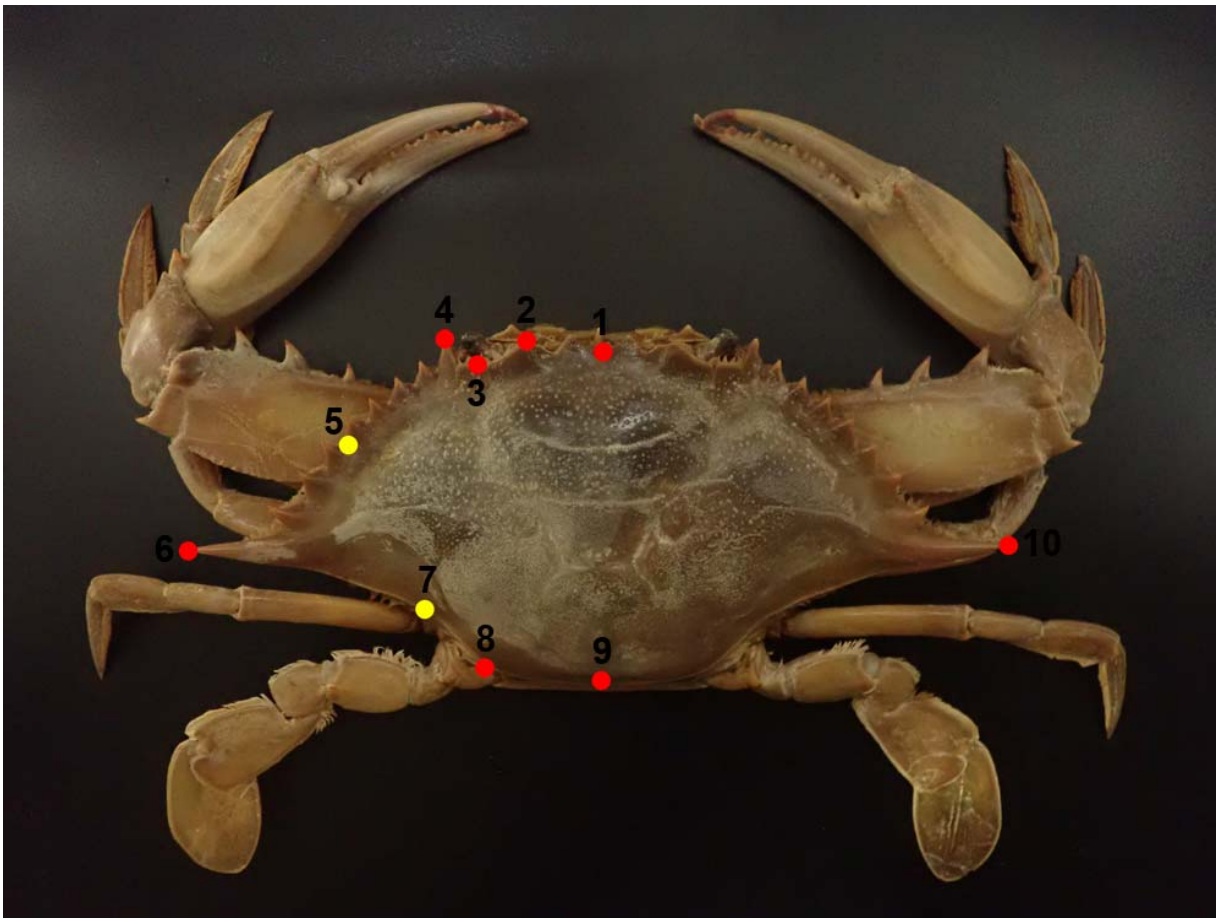
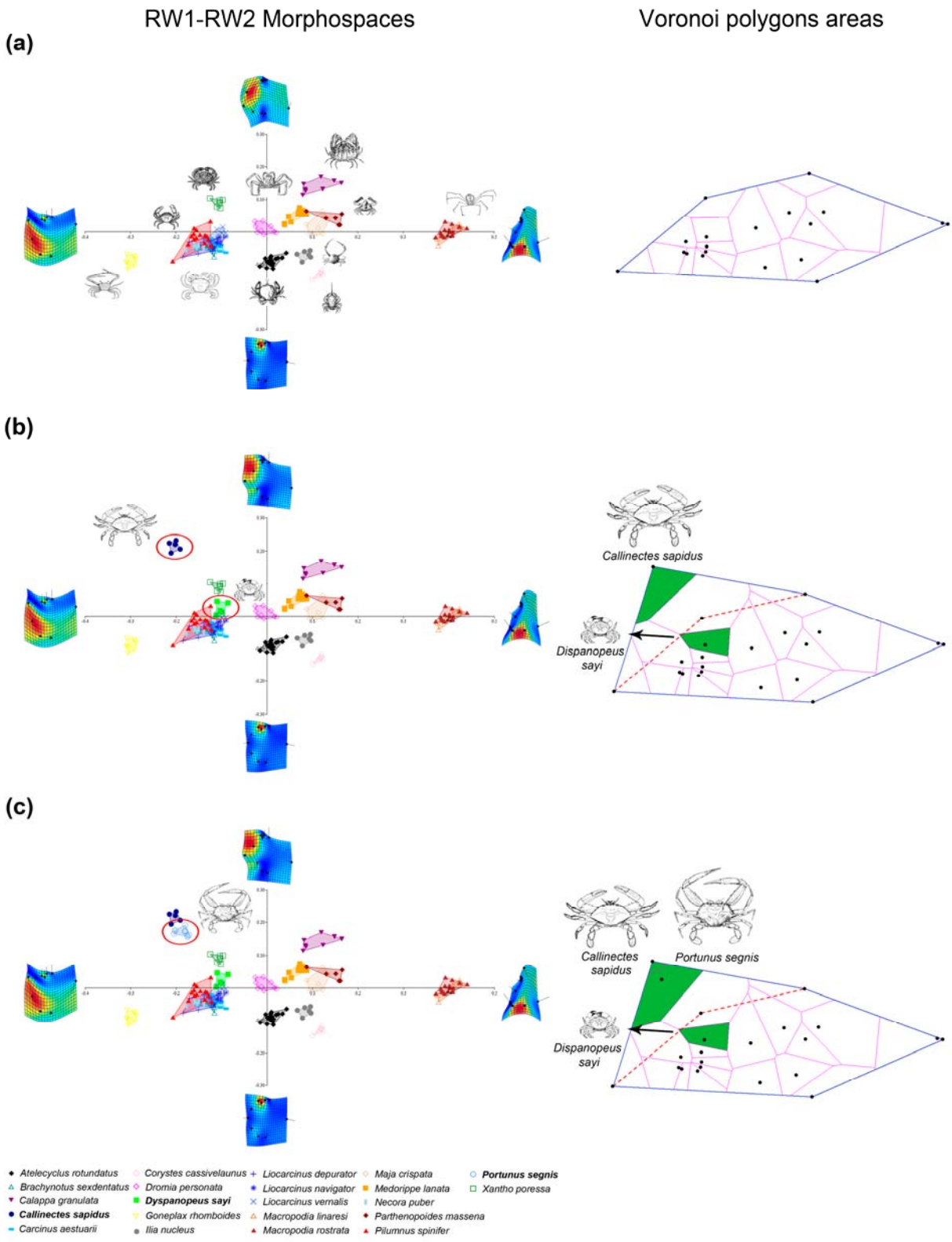


Fig. 2 Scheme of the landmarks (N=8, red points) and semilandmarks (N=2, yellow points) used for description of the dorsal carapace shape of crabs. The definition of the meaning of landmarks and semilandmarks is detailed in Supplementary material, Table S1. The specimen of the image is an individual of *Callinectes sapidus* from the Biological Reference Collections (CBR) of the Marine Science Institute (ICM-CSIC) of Barcelona


RW1-RW2 Morphospaces
Voronoi polygons areas
(a)
(b)
(c)
Callinectes sapidus
Dispanopeus sayi
Callinectes sapidus
Portunus segnis
Dispanopeus sayi
Atelecyclus rotundatus
Brachynotus sexdentatus
Calappa granulata
Callinectes sapidus
Carcinus aestuarii
Corystes cassivelaunus
Dromia personata
Dyspanopeus sayi
Goneplax rhomboides
Ilia nucleus
Liocarcinus depurator
Liocarcinus navigator
Liocarcinus vernalis
Macropodia linaresi
Macropodia rostrata
Maja crispata
Medorippe lanata
Necora puber
Parthenopoides massena
Pilumnus spinifer
Portunus segnis
Xantho poressa

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765 **Fig. 3** Two-dimensional (RW1-RW2) morphospace representations and Voronoi polygons
766 representation of the **a** native (NCC), **b** current (CCC) and **c** future crab community (FCC) of
767 Alfacs Bay (Ebro Delta, NW Mediterranean). In the morphospaces, the different convex hulls
768 enclose all specimens of each species, and the color range of symbols is related with the
769 taxonomical similarity (superfamily) of species. Deformation grids indicating the shape variation
770 along the axes and a draw of most representative species are also provided. The position of the
771 non-native species (in bold in legend) in CCC and FCC is highlighted by a red circle. In the
772 Voronoi polygons representation, the position and occupied area by the non-native species is
773 colored in green. The red dashed line represents the original outline of the native morphospace,
774 underlying the increase of the morphological space produced by the non-native species

Table 1 Specific composition of the crab assemblage inhabiting the Alfacs Bay (Ebro Delta, NW Mediterranean), including native and alien species, for the three assessed temporal moments: native crab community (NCC), current crab community (CCC) and future crab community (FCC). ID, identification code; N, number of adult males individuals used for the morphological analysis; NND, the nearest-neighbor distance; VOR, Voronoi polygon area; MD, morphological disparity. Species changing its NND or VOR by invasion of new crabs are marked in bold. The taxonomical classification (superfamily) of each species is provided

Origin	Superfamily	Species	ID	N	Native Crab Community (NCC)			Current Crab Community (CCC)			Future Crab Community (FCC)		
					NND (ID)	VOR	MD	NND (ID)	VOR	MD	NND (ID)	VOR	MD
Native	Calappoidea	*Calappa granulata*	3	8	0.099 (17)	5883.0	0.0002	0.099 (17)	**9637.0**	0.0002	0.099 (17)	9637.0	0.0002
	Cancroidea	*Atelecyclus rotundatus*	1	19	0.077 (10)	12375.0	0.0009	0.077 (10)	12375.0	0.0009	0.077 (10)	12375.0	0.0009
	Corystoidea	*Corystes cassivelaunus*	6	6	0.068 (10)	9230.0	0.0004	0.068 (10)	9230.0	0.0004	0.068 (10)	9230.0	0.0004
	Dorippoidea	*Medorippe lanata*	17	14	0.057 (16)	13853.0	0.0013	0.057 (16)	**15262.0**	0.0013	0.057 (16)	15262.0	0.0013
	Dromioidea	*Dromia personata*	7	9	0.084 (17)	18862.5	0.0005	0.084 (17)	**18659.0**	0.0005	0.084 (17)	18659.0	0.0005
	Goneplacoidea	*Goneplax rhomboides*	9	9	0.150 (18)	3681.1	0.0009	0.150 (18)	**8575.5**	0.0009	0.150 (18)	**8559.5**	0.0009
	Grapsoidea	*Brachynotus sexdentatus*	2	5	0.010 (5)	4986.6	0.0011	0.010 (5)	4986.6	0.0011	0.010 (5)	4986.6	0.0011
	Leucosioidea	*Ilia nucleus*	10	6	0.067 (6)	11609.5	0.0013	0.067 (6)	11609.5	0.0013	0.067 (6)	11609.5	0.0013
	Majoidea	*Macropodia linaresi*	14	7	0.006 (15)	1.2	0.0042	0.006 (15)	1.2	0.0042	0.006 (15)	1.2	0.0042
	Majoidea	*Macropodia rostrata*	15	16	0.006 (14)	14097.5	0.0018	0.006 (14)	14097.5	0.0018	0.006 (14)	14097.5	0.0018
	Majoidea	*Maja crispate*	16	7	0.039 (19)	8682.0	0.0027	0.039 (19)	8682.0	0.0027	0.039 (19)	8682.0	0.0027
	Parthenopoidea	*Parthenopoides massena*	19	6	0.039 (16)	30054.5	0.0009	0.039 (16)	30054.5	0.0009	0.039 (16)	30054.5	0.0009
	Pilumnoidea	*Pilumnus spinifer*	20	15	0.028 (18)	6521.5	0.0017	0.028 (18)	**16642.0**	0.0017	0.028 (18)	**15056.8**	0.0017
	Portunoidea	*Carcinus aestuarii*	5	7	0.011 (2)	3182.3	0.0008	0.011 (2)	3182.3	0.0008	0.011 (2)	3182.3	0.0008
	Portunoidea	*Liocarcinus navigator*	12	7	0.016 (5)	2361.2	0.0007	0.016 (5)	2361.2	0.0007	0.016 (5)	2361.2	0.0007
	Portunoidea	*Liocarcinus depurator*	11	10	0.018 (18)	2913.7	0.0024	0.018 (18)	2913.7	0.0024	0.018 (18)	2913.7	0.0024
	Portunoidea	*Liocarcinus vernalis*	13	7	0.023 (12)	7412.0	0.0003	0.023 (12)	**2887.1**	0.0003	0.023 (12)	2887.1	0.0003
	Portunoidea	*Necora puber*	18	6	0.018 (11)	7850.8	0.0004	0.018 (11)	**8015.3**	0.0004	0.018 (11)	8015.3	0.0004
	Xanthoidea	*Xantho poressa*	22	6	0.098 (13)	7671.5	0.0018	**0.067 (8)**	**33399.5**	0.0018	0.067 (8)	**27427.0**	0.0018
Non-native	Portunoidea	*Callinectes sapidus*	4	5	-	-	-	0.1579 (22)	12332.5	0.0012	**0.0486 (4)**	**830.4**	0.0012
	Xanthoidea	*Dyspanopeus sayi*	8	5	-	-	-	0.0322 (13)	7808.0	0.0002	0.0322 (13)	7808.0	0.0002
	Portunoidea	*Portunus segnis*	21	9	-	-	-	-	-	-	0.0486 (4)	18746.5	0.0004

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Table S1 Description of the meaning of landmarks and semilandmarks (shown in Fig. 2) used to characterize the dorsal shape of the carapace of the analyzed brachyuran crabs. The references of the consulted bibliography to define the landmarks and semilandmarks are provided

# point	Type of point	Definition	Bibliography used
1	Landmark	Middle point of the outer frontal margin of the carapace	Rufino et al. 2006, Sanchez et al. 2013, Alencar et al. 2014, Duarte et al. 2014, Torres et al. 2014
2	Landmark	Inner tip of the orbital depression	Idazskin et al. 2013, Sanchez et al. 2013, Duarte et al. 2014, Torres et al. 2014
3	Landmark	Deepest point of the orbital depression	Idazskin et al. 2013, Sanchez et al. 2013, Silva et al. 2014
4	Landmark	Intersection of anterior and anterolateral margin of the carapace, or outer tip of the orbital depression	Rufino et al. 2006, Idazskin et al. 2013, Alencar et al. 2014, Duarte et al. 2014, Silva et al. 2014, Torres et al. 2014, Hopkins and Thurman 2010
5	Semilandmark	Middle point along the anterolateral margin of the carapace between landmark 4 and 6	Rufino et al. 2006, Idazskin et al. 2013, Sanchez et al. 2013, Silva et al. 2014, Torres et al. 2014
6	Landmark	Intersection between the anterolateral and posterolateral margin of the carapace, or tip of the last anterolateral spine	Rufino et al. 2006, Sánchez et al. 2013, Alencar et al. 2014, Duarte et al. 2014, Hopkins and Thurman 2010
7	Semilandmark	Middle point along the posterolateral margin between landmark 6 and 8	Hopkins and Thurman 2010, Idazskin et al. 2013, Sanchez et al. 2013, Torres et al. 2014
8	Landmark	Maximum curvature of the posterior margin of the carapace	Rufino et al. 2006, Hopkins and Thurman 2010, Sanchez et al. 2013, Silva et al. 2014, Torres et al. 2014
9	Landmark	Middle point of the outer posterior margin of the carapace	Rufino et al. 2006, Hopkins and Thurman 2010, Sanchez et al. 2013, Alencar et al. 2014, Duarte et al. 2014, Silva et al. 2014, Torres et al. 2014
10	Landmark	Tip of the last anterolateral spine of the right side of the carapace, indicating the maximum width of the carapace along with landmark 6	Rufino et al. 2006, Hopkins and Thurman 2010, Idazskin et al. 2013, Sanchez et al. 2013, Silva et al. 2014

References

Alencar CERD, Lima-Filho PA, Molina WF, Freire FAM (2014) Sexual shape dimorphism of the mangrove crab *Ucides cordatus* (Linnaeus, 1763) (Decapoda, Ucididae) accessed through geometric morphometric. Sci World J 2014:206168. doi: 10.1155/2014/206168

Duarte RC, Ré A, Flores AAV, Queiroga H (2014) Conspecific cues affect stage-specific molting frequency, survival, and claw morphology of early juvenile stages of the shore crab *Carcinus maenas*. Hydrobiologia 724:55–66. doi: 10.1007/s10750-013-1712-5

Hopkins MJ, Thurman CL (2010) The geographic structure of morphological variation in eight species of fiddler crabs (Ocypodidae: genus *Uca*) from the eastern United States and Mexico. Biol J Linn Soc 100:248–270

Idaszkin YL, Márquez F, Nocera AC (2013) Habitat-specific shape variation in the carapace of the crab *Cyrtograpsus angulatus*. J Zool 290:117–126

Rufino MM, Abelló P, Yule AB (2006) Geographic and gender shape differences in the carapace of *Liocarcinus depurator* (Brachyura: Portunidae) using geometric morphometrics and the influence of a digitizing method. J Zool 269:458–465. doi: 10.1111/j.1469-7998.2006.00086.x

Sanchez MLS, Gorospe JG, Gorospe JN, Torres MAJ, Demayo CG (2013) Describing geographic differences in carapace shape in the blue swimming crab *Portunus pelagicus* from Mindanao Bays, Philippines. AACL Bioflux 6:622–634

Silva IC, Alves MJ, Paula J, Hawkins SJ (2014) Population differentiation of the shore crab *Carcinus maenas* (Brachyura: Portunidae) on the southwest English coast based on genetic and morphometric analyses. Sci Mar 74(3):435–444

Torres MV, Collins PA, Giri F (2014) Morphological variation of freshwater crabs *Zilchiopsis collastinensis* and *Trichodactylus borellianus* (Decapoda, Trichodactylidae) among localities from the middle Paraná River basin during different hydrological periods. ZooKeys 457:171–186